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APPLICATION FILED

Patents ADP number *(if you know it)* 00773762001

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention — TREATMENT OF BACTERIAL INFECTIONS

5. Name of your agent *(if you have one)* — Marks & Clerk

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Treatment of Bacterial Infections

The present invention relates to polypeptides, derivatives or analogues thereof, with antibacterial activity, and to nucleic acids encoding the same. The invention further provides the use of such polypeptides, derivatives, analogues or nucleic acids as medicaments, and also in methods of treatment.

Antimicrobial peptides are a key component of the innate immune system, generally containing 20-40 amino acids, having a net positive charge, and with the majority having been identified so far in non-mammalian species. The latter limits their usefulness as therapeutics in humans or mammals, both due to difficulties in commercial production of such large peptides, and due to the risk of adverse effects of these peptides due to their non-human origin. By 2003, of the around 800 sequences listed in the Trieste international antimicrobial peptide database (http://www.bbcm.units.it/~tossi/amsdb.html), only 33 were of human origin, and of these only 3 are less than 20 amino acids in length. Some short synthetic antimicrobial peptides have also been developed. However, these have the disadvantage of associated risks of antigenic or toxic effects due to their non-human origin.

Such peptides have been characterised into six groups (Bradshaw, J.P., Biodrugs, 2003: 17: 235-240), with the following three classes being most studied (Bowman H.G., Journal of Internal Medicine, 2003: 254:197-215):

Linear peptides lacking cysteines and often with an α-helical amphipathic structure in solution, for example, Human LL-37 (SEQ ID No.19):- LLGDFFRKSKEKIGKEFKRI VQRIKDFLRN LVPRTES;

(i) Peptides with 3 disulphide bonds, giving peptides with a flat dimeric beta-sheet, for example, Human α-defensin:- HNP-1 (SEQ ID No.20)

ACYCRIPACI AGERRYGTCI YQGRLWAFCC

(ii) Peptides with unusual bias in certain amino acids such as proline, arginine, tryptophan or histidine, for example, Pig PR-39 (SEQ ID No.21):-

RRRPRPPYLP RPRPPPFFPP RLPPRIPPGF PPRFPPRFP; or cow indolicidin (SEQ ID No.22):- ILPWKWPWWP WRR.

Many antimicrobial peptides have the capacity to lyse bacteria. However, it is unclear whether cell lysis is the mechanism responsible for such antimicrobial effects (Bowman *supra*). In addition, many antibacterial peptides have a net positive charge, and have an element of amphipathicity with a hinge that could help the peptide to flip into a bacterial membrane. However, both of these features are common to many peptides, including, for example, polypeptide hormones (Bowman *supra*). Hence, at present, the mechanism by which antimicrobial peptides impart their antimicrobial function is currently not fully understood. Although some have been found to exert antiviral action also, this is considered to be a minor side effect of any membrane disrupting action of these peptides (Bowman *supra*).

A number of antibacterial peptides that have been described in the scientific literature have strong cationic character, and often consist of arginine and lysine residues. However, not all peptides containing arginine and lysine have antimicrobial activity. For example, Azuma *et al.* (Peptides, 21: 327-330 (2000)) reported that a 30-mer monomeric peptide consisting of $apoE_{133-162}$ (LRVRLASHLRKLRKRLL RDADDLQKRLAVY) had antimicrobial activity, which was comparable with that of the antibiotic gentamicin. However, the authors found that substituting individual arginines at positions 136, 142, 147 and 150 of the peptide diminished antibacterial activity, with residues at 142 and 147 appearing to be the most crucial. In addition, as Azuma designed shorter peptides approaching this region, the antibacterial activity declined. For example, Azuma's peptide $apoE_{134-151}$ had no activity at all despite containing arginines both at positions 142 and 147. Similarly, Azuma demonstrated that the peptide $apoE_{134-155}$ had very low antibacterial activity, and the peptide $apoE_{134-159}$ had greatly reduced antibacterial activity.

Therefore, it appears that Azuma *et. al.* had no means to harness the potential antibacterial activity of their $apoE_{133-162}$ peptide, and demonstrated that simply constructing a peptide, which was cationic, did not necessarily guarantee that it would exhibit antibacterial efficacy. Hence, the mechanism by which Azuma's peptide imparted it's antibacterial nature was not at all clear.

The inventors of the present invention have investigated the antiviral action of peptides comprising tandem repeats of $apoE_{141-149}$ (LRKLRKRLL – SEQ ID No.1) and $apoB_{3359-3367}$ (RLTRKRGLK – SEQ ID No.2). These peptides are either derived from or comprise the LDL receptor / HSPG receptor binding region of these apolipoproteins, and they were constructed as tandem repeats of SEQ ID No.1 or SEQ ID No.2, or closely related sequences. While the inventors do not wish to be bound by any hypothesis, they consider it likely that these peptides exert their antiviral actions by a number of mechanisms, with those affecting viral attachment being particularly favoured.

The inventors have found that stabilisation of peptides derived from the LDL receptor / HSPG receptor binding region of these apolipoproteins, as a tandem repeat peptide is important for its antiviral effects. Such stabilisation has been found to increase the ability of the peptide to form an alpha helix in the presence of organic membrane mimicking solvent (50% TFE).

The inventors have also investigated the lytic activity of these tandem repeat peptides, and examined whether there was any evidence for a direct lytic effect of the $apoE_{141-149}$ tandem repeat peptide on HIV. They have found that direct treatment of HIV with the $apoE_{141-149}$ tandem repeat peptide alone does not lyse the virus, and therefore has no effect on viral replication. Hence, it appears that the tandem repeat peptide of $apoE_{141-149}$ does not have any function as a lytic peptide. As a result, although the inventors do not wish to be bound by any hypothesis, they postulate that the tandem repeat exerts its antiviral effect by blocking viral attachment to cells instead of by viral lysis.

Despite the fact that antiviral agents are unrelated to antibacterial agents due to their different modes of action on viruses and bacteria, respectively, the inventors decided to investigate whether the peptides had any unrelated anti-bacterial properties. To this end, they have investigated whether lytic or other effects exhibited by the peptides are possible with bacteria, even though these seemed not to occur with viruses. Specifically, the inventors wondered whether construction of a tandem repeat of the $apoE_{141-149}$ region might unexpectedly allow the antibacterial properties of

$apoE_{133-162}$ disclosed in Azuma *et. al.* to be recaptured within a much shorter peptide. In addition, the inventors have also investigated the antibacterial efficacy of peptides derived from $apoB_{3359-3367}$.

The inventors first investigated the antibacterial efficacy of two peptides GIN 2 and GIN 11, which did not show any antiviral activity.

According to a first aspect of the present invention, there is provided a peptide, derivative or analogue thereof comprising an amino acid sequence of SEQ ID No.3 (GIN 2) or SEQ ID No.4 (GIN 11), for use as a medicament.

To the inventor's surprise, when these two peptides in accordance with the first aspect were tested on bacteria, they did show antibacterial efficacy, as shown in Table 2 of the Example. Hence, it is the inventor's belief that they have demonstrated the first medical indication for these two peptides.

The monomer peptide of $apoE_{141-149}$ of SEQ ID No.1: LRKLRKRLL (i.e. a 9-mer) is repeated to produce a tandem repeat of $apoE_{141-149}$ of SEQ ID No. 6, having the amino acid sequence: LRKLRKRLLLRKLRKRLL, i.e. an 18-mer. SEQ ID No.3 is a peptide corresponding to the full length tandem repeat of $apoE_{141-149}$ (SEQ ID NO. 6) truncated by the excision of amino acids 1, 2 and 3, i.e. it is a 15-mer. This peptide is designated GIN 2 when referred to herein.

SEQ ID No.4 is a peptide comprising $apoE_{128-149}$ having an amino acid sequence of QSTEELRVRLASHLRKLRKRLL, and is designated GIN 11, when referred to herein.

The inventors then investigated the antibacterial efficacy of several peptides, which did show antiviral activity.

According to a second aspect of the present invention, there is provided use of a tandem repeat of a peptide, derivative or analogue thereof, which peptide is derived from a Heparan Sulphate Proteoglycan (HSPG) receptor binding region of

apolipoprotein B or apolipoprotein E, or a truncation thereof, for the manufacture of a medicament for the treatment of a bacterial infection.

The inventors have found that peptides as defined above have antiviral activity. To the inventor's surprise, when the peptides according to the second aspect were tested on bacteria, they also showed antibacterial efficacy, as shown in the Example. Hence, it is the inventor's belief that they have therefore shown a new medical indication for these peptides.

The medicament may be used for the treatment of a variety of bacterial infections, including: microbial keratitis; conjunctivitis; bronchopulmonary infections, for example, pneumonia; urinary tract infections, for example, cystitis, pyelonephritis; ear, nose, and throat infections, for example, otitis media, sinusitis, laryngitis, diphtheria; skin infections including cellulitis, impetigo, wound infections, botulism, gonorrhoea; Septicaemia; peptic and duodenal ulcer; gastritis; *Campylobacter* infections; *Proteus mirabilis* infections; meningitis; osteomyelitis; and Salmonellosis.

In general, antiviral agents, such as acyclovir, ribavirin, or enfuvirtide (T-20), are rarely useful against bacterial infections due to their completely different modes of action. Similarly, antibacterial agents, such as antibiotics, are rarely useful against viral infections. Accordingly, the inventors were very surprised that the peptides according to the second aspect showed both antiviral and antibacterial efficacy, as this was completely unexpected.

It is preferred that the peptide according to the second aspect of the invention is derived from an apolipoprotein B LDL receptor binding domain cluster B, as defined by Law and Scott (J. Lipid Res. 1990, 31:1109-20), or from the LDL receptor binding region of apoE (J. Lipid Res. 1995, 36:1905-1918). The LDL receptor binding domain cluster B may be located within an HSPG receptor binding region of apolipoprotein B, and the LDL receptor binding region of apolipoprotein E may be located within an HSPG binding domain of apoE.

The term "derived" as used herein is intended to describe or include a peptide, which is a derivative or a modification of an amino acid sequence forming the HSPG

receptor binding region of apoB or apoE, or the LDL receptor binding domain cluster B of apoB or LDL receptor binding domain of apoE. Suitable modification may include amino acid substitution, addition or deletion. Preferably, the derivative peptide or modified peptide is arranged as a tandem repeat in accordance with the second aspect of the invention.

The inventors conducted exhaustive experiments to assess the antibacterial activity of peptides from apolipoproteins and derivatives thereof. Peptides and derivatives from ApoE and ApoB were a particular focus. The inventors found that the $apoE_{141-149}$ monomeric sequence (SEQ ID No.1) and the $apoB_{3359-3367}$ (SEQ ID No. 2) had no detectable antibacterial activity. In addition, to the inventor's surprise they found that a large number of other related peptides had little or no antibacterial effect (see Example 1, Tables 1 and 2).

However, surprisingly, the inventors found that peptides in accordance with the first and second aspects of the present invention, do exhibit antibacterial activity. Example 1 illustrates the efficacy of the peptides according to the invention compared to $apoE_{141-149}$ and $apoB_{3359-3367}$ (i.e. non-tandem repeats) and other peptides derived from apolipoproteins.

While the inventors do not wish to be bound by any hypothesis, the inventors believe that the cationic amino acid residues in both the $apoE_{141-149}$ peptides and $apoB_{3359-3367}$ peptides are stabilised within a form in peptides according to the invention (i.e. tandem repeats), which allows comparable anti-bacterial activity to that reported for the Azuma peptide ($apoE_{133-162}$). The inventors have also established that certain derivatives of these peptides also have antibacterial activity, including modifications and truncations of the peptide sequences.

In one embodiment, the peptide according to the second aspect may comprise a tandem repeat of $apoE_{133-162}$ of SEQ ID NO. 5, or a truncation thereof.

By "a tandem repeat of $apoE_{133-162}$ of SEQ ID No. 5", we mean the peptide with the amino acid sequence: LRVRLASHLRKLRKRLLRDADDLQKRLAVY LRVRLASHLRKLRKRLLRDADDLQKRLAVY. The $apoE_{133-162}$ peptide disclosed

in Azuma *et al.* was shown to have some antibacterial activity. However, surprisingly, the inventors of the present invention have shown that a tandem repeat of Azuma's peptide has improved antibacterial efficacy.

In a preferred embodiment, the peptide according to the second aspect may comprise a tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6, or a truncation thereof.

By "a tandem repeat of $apoE_{141-149}$ of SEQ ID No. 6", we mean the peptide with the amino acid sequence: LRKLRKRLLLRKLRKRLL, i.e. an 18-mer. Hence, the tandem repeat of $apoE_{141-149}$ of SEQ ID No. 6, preferably comprises a repeat of the amino acid sequence: LRKLRKRLL, i.e. a 9-mer. The tandem repeat of $apoE_{141-149}$ of SEQ ID No. 6 is referred to herein as GIN 1 or GIN1p (when N terminal protected by an acetyl group, and C terminal protected by an amide group).

Preferably, the peptide according to the second aspect comprises a tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6, characterised in that at least one Leucine (L) residue of SEQ ID No. 6 is replaced by a Tryptophan (W) or Tyrosine (Y) residue or derivatives thereof.

It is preferred that the polypeptide according to the second aspect of the invention has at least two W or Y substitutions, and more preferably three or more W or Y substitutions.

The substituted polypeptide may comprise 18 amino acids (or derivatives thereof) and thereby correspond to the full length of SEQ ID No. 6. However, the inventors have surprisingly found that some selected truncated peptides based on SEQ ID No.6 also have efficacy as antibacterial agents. Accordingly, preferred peptides or derivatives thereof may have less than 18 amino acids. For instance, some peptides according to the second aspect of the invention may be 17, 16, 15, 14, 13, 12, 11, 10 or less amino acids in length.

It will be appreciated that modified forms of W or Y may be substituted into the tandem repeat of $apoE_{141-149}$. Such peptides will still have antibacterial activity provided that the modification does not significantly alter its chemical characteristics.

Preferred peptides according to the second aspect of the invention may comprise one of the following amino acid sequences:-

(a) WRKWRKRWWWRKWRKRWW (SEQ ID No. 7). This peptide corresponds to the full length tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6 with all Leucines substituted for Tryptophan residues, i.e. is an 18-mer. This peptide is designated GIN 7 when referred to herein.

(b) WRKWRKRWRKWRKR (SEQ ID No. 8). This peptide corresponds to the full length tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6 with all Leucines substituted for Tryptophan residues and truncated by the excision of amino acids 9, 10, 17 and 18, i.e. is a 14-mer. This peptide is designated GIN 32 when referred to herein.

(c) WRKWRKRWWLRKLRKRLL (SEQ ID No. 9). This peptide corresponds to the full length tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6 with a subset of Leucines substituted for Tryptophan residues, i.e. is an 18-mer. This peptide is designated GIN 34 when referred to herein.

(d) YRKYRKRYYYRKYRKRYY (SEQ ID No. 10). This peptide corresponds to the full length tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6 with all Leucines substituted for tyrosine residues, i.e. is an 18-mer. This peptide is designated GIN 41 when referred to herein.

(e) LRKLRKRLRKLRKR (SEQ ID No. 11). This peptide corresponds to the full length tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6 truncated by the excision of amino acids 9, 10, 17 and 18, i.e. is an 14-mer. This peptide is designated GIN 8 when referred to herein.

(f) LRKRLLLRKLRKRLL (SEQ ID No.3). This peptide corresponds to the full length tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6 truncated by the excision of amino acids 1, 2 and 3, i.e. is a 15-mer. This peptide is designated GIN 2 when referred to herein.

The peptide according to the second aspect may comprise a tandem repeat of $apoB_{3359-3367}$ of SEQ ID No. 12, or a truncation thereof.

By "a tandem repeat of $apoB_{3359-3367}$ SEQ ID No. 12", we mean the peptide with the amino acid sequence: RLTRKRGLKRLTRKRGLK.

Preferably, the peptide according to the second aspect comprises a tandem repeat of $apoB_{3359-3367}$ of SEQ ID No.12 or a truncation thereof, characterised in that at least one amino acid residue has been replaced by a Tryptophan (W), Arginine (R) or Leucine (L) residue or derivative thereof.

Suitably, one or more, more suitably, two or more, and even more suitably, three or more amino acid residues may be replaced by a Tryptophan (W), Arginine (R) or Leucine (L) residue or derivative thereof. Preferably, four or more, more preferably, five or more, and even more preferably, six or more amino acid residues may be replaced by a Tryptophan (W), Arginine (R) or Leucine (L) residue or derivative thereof. Preferably, the replaced or substituted residue is the first, second, third, seventh, eighth, and/or ninth residue of the repeated amino acid sequence of $apoB_{3359-3367}$, or combinations thereof.

Preferred peptides according to the second aspect of the invention comprise one of the following amino acid sequences:-

(a) RTRKRGRRTRKRGR (SEQ ID No.13). This peptide is designated GIN 36 when referred to herein;

(b) LRKRKRLLRKRKRL (SEQ ID No.14). This peptide is designated GIN 37 when referred to herein;

(c) LRKRKRLRKLRKRKRLRK (SEQ ID No.15). This peptide is designated GIN 38 when referred to herein; and

(d) WRWRKRWRKWRWRKRWRK (SEQ ID No.16). This peptide is designated GIN 33 when referred to herein.

According to a third aspect of the present invention, there is provided use of a peptide comprising $apoE_{128-149}$ of SEQ ID NO.2, or a truncation thereof, for the manufacture of medicament for the treatment of a bacterial infection.

The peptide according to the third aspect has the amino acid sequence: QSTEELRVRLASHLRKLRKRLL. This peptide is also referred to herein as GIN 11.

Due to their increased biological activity, polypeptides, derivatives or analogues according to the invention are of utility as antibacterial agents.

Polypeptides, derivatives or analogues according to the invention may be used in the treatment against any bacterium, or bacterial infection. The bacterium may be a gram positive or a gram negative bacterium.

For example, bacteria against which the peptides in accordance with the invention are effective may include *Firmicutes*, which may be *Bacilli* or *Clostridia*, for example *Clostridium botulinum*.

In a preferred embodiment, bacteria against which the peptides in accordance with the invention are effective may include *Bacillales*, preferably, *Staphylococcus*, for example, *Staphylococcus aureus*. Additional *Bacillales* with which the peptides according to the invention are effective include *Streptococci*, for example, *Streptococcus pyogenes*.

Further examples of bacteria against which the peptides in accordance with the invention are effective may include *Pseudomonadales*, preferably, *Pseudomonas aeruginosa*. Further examples of bacteria against which the peptides in accordance with the invention are effective may include *Gammaproteobacteria*, which may be independently selected from a group consisting of *Enterobacteriales*, *Proteus*, *Serratai*, *Pasteurellales*, and *Vibrionales*. Preferred *Enterobacteriales* include *Escherichia*. Preferred *Proteus* include *Proteus mirabilis*. Preferred *Serratai* include *Serratia marcescens*. Preferred *Pasteurellales* include *Haemophilus influenzae*. Preferred *Vibrionales* include *Vibrio cholerae*.

Further examples of bacteria against which the peptides in accordance with the invention are effective may include *Betaproteobacteria*, including *Neisseriales*, for example, *Neisseria gonorrhoeae.* Further examples of bacteria against which the peptides in accordance with the invention are effective may include *Delta/epsilon subdivided Proteobacteria*, including *Campylobacterales*, for example *Helicobacter pylori.* Further examples of bacteria against which the peptides in accordance with the invention are effective may include *Actinobacteria*, for example *Mycobacterium tuberculosis and Nocardia asteroides.*

The inventors conducted experiments to determine which of the peptides disclosed herein exhibited antibacterial activity against the test bacteria *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The activity of peptides in accordance with the invention can be seen in Tables 1 and 2. Preferably, the peptides according to the invention exhibit antibacterial activity against both *S. aureus*, and *P. aeruginosa*. However, the inventors have found that GIN 41 (SEQ ID No.10) is particularly active against *Staphylococcus*, and in particular, *S.aureus*. In addition, the inventors have found that GIN 8 (SEQ ID No.11), GIN 2 (SEQ ID No.3), and GIN 11 (SEQ ID No.4) are particularly effective against *Pseudomonadales*, and in particular, *P.aeruginosa*.

Unlike the antiviral activity of the $apoE_{141-149}$ peptides, where several mechanisms, which involve prevention of virus from attaching to a cell or possibly from fusing with the cell membrane are likely to operate, in an antibacterial assay, the inventors believe that cell entry can not take place. Hence, whilst the inventors do not wish to be bound by any hypothesis, they have suggested that the antibacterial mechanism of action by the peptides in accordance with the invention, may involve a direct damaging effect to the bacterium, either mediated through the bacterial membrane, or through targeting a site within the bacterium. It is possibly for this reason that only a surprisingly small number of peptide sequences have been found to be effective against bacteria.

It will be appreciated that the therapeutic effects of polypeptides, derivatives or analogues according to the invention may be mediated "indirectly" by agents that increase the activity of such polypeptides, derivatives or analogues.

Thus, according to a fourth aspect of the invention, there is provided an agent capable of increasing the biological activity of a polypeptide, derivative or analogue according to the first, second or third aspect of the invention for use as a medicament.

Agents capable of increasing the biological activity of polypeptides, derivatives or analogues according to the invention may achieve their effect by a number of means. For instance, such agents may increase the expression of such polypeptides, derivatives or analogues. Alternatively (or in addition), such agents may increase the half-life of polypeptides, derivatives or analogues according to the invention in a biological system, for example, by decreasing turnover of the polypeptides, derivatives or analogues.

Polypeptides, derivatives or analogues according to the invention may be used to treat bacterial infections as a monotherapy (i.e. use of the peptide or nucleic acid alone) or in combination with other compounds or treatments used in antibacterial therapy. For example, conventional antibiotics include amikacin, amoxicillin, aztreonam, cefazolin, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, linezolid, nafcillin, piperacillin, quinopristin-dalfoprisin, ticarcillin, tobramycin, and vancomycin.

Hence, in accordance with a further aspect of the invention, there is provided use of an agent capable of increasing the biological activity of a polypeptide, derivative or analogue according to the first or second or third aspect of the invention for the manufacture of a medicament for treating a bacterial infection.

Derivatives of polypeptides according to the invention may include derivatives that increase or decrease the polypeptide's half-life *in vivo*. Examples of derivatives capable of increasing the half-life of polypeptides according to the invention include peptoid derivatives of the polypeptides, D-amino acid derivatives of the polypeptides, and peptide-peptoid hybrids.

Polypeptides according to the invention may be subject to degradation by a number of means (such as protease activity in biological systems). Such degradation may limit the bioavailability of the polypeptides and hence the ability of the polypeptides to achieve their biological function. There are wide ranges of well-established techniques by which peptide derivatives that have enhanced stability in biological contexts can be designed and produced. Such peptide derivatives may have improved bioavailability as a result of increased resistance to protease-mediated degradation. Preferably, a peptide derivative or analogue suitable for use according to the invention is more protease-resistant than the peptide from which it is derived.

Preferably, the peptide may be made more protease-resistant by protecting the N and/or C terminal. For example, the N terminal may be protected by an acetyl group. The C terminal may be protected by an amide group.

Protease-resistance of a peptide derivative and the peptide from which it is derived may be evaluated by means of well-known protein degradation assays. The relative values of protease resistance for the peptide derivative and peptide may then be compared.

Peptoid derivatives of the peptides of the invention may be readily designed from knowledge of the structure of the peptide according to the first, second or third aspect of the invention. Commercially available software may be used to develop peptoid derivatives according to well-established protocols.

Retropeptoids, (in which all amino acids are replaced by peptoid residues in reversed order) are also able to mimic antiviral peptides derived from apolipoproteins. A retropeptoid is expected to bind in the opposite direction in the ligand-binding groove, as compared to a peptide or peptoid-peptide hybrid containing one peptoid residue. As a result, the side chains of the peptoid residues are able to point in the same direction as the side chains in the original peptide.

A further embodiment of a modified form of polypeptide according to the invention comprises D-amino acid forms of the polypeptide. In this case, the order of

the amino acid residues is reversed. The preparation of peptides using D-amino acids rather than L-amino acids greatly decreases any unwanted breakdown of such an agent by normal metabolic processes, decreasing the amounts of agent which need to be administered, along with the frequency of its administration.

The polypeptides, analogues, or derivatives of the invention represent products that may advantageously be expressed by biological cells.

The present invention also provides, in a fifth aspect, a nucleic acid sequence encoding a polypeptide, derivative or analogue according to the invention.

Preferred nucleic acids according to the fifth aspect of the invention may include SEQ ID No.25, SEQ ID No.26, SEQ ID No.27, SEQ ID No.28, SEQ ID No.29, SEQ ID No.30, SEQ ID No.31, SEQ ID No.32, SEQ ID No.33, SEQ ID No.34, SEQ ID No.35, SEQ ID No.36, SEQ ID No.37, and SEQ ID No.38.

It will be appreciated that, due to redundancy in the genetic code, a nucleic acid sequence in accordance with the fifth aspect of the invention may vary from the naturally occurring ApoB or ApoE genes providing a codon encodes a polypeptide, derivative or analogue thereof in accordance with the first, second or third aspect of the invention.

By the term "derivative", or "modification", we mean that the sequence has at least 30%, preferably 40%, more preferably 50%, and even more preferably, 60% sequence identity with the amino acid/polypeptide/nucleic acid sequences of any of the sequences referred to herein. An amino acid/polypeptide/nucleic acid sequence with a greater identity than preferably 65%, more preferably 75%, even more preferably 85%, and even more preferably 90% to any of the sequences referred to is also envisaged. Preferably, the amino acid/polypeptide/nucleic acid sequence has 92% identity, even more preferably 95% identity, even more preferably 97% identity, even more preferably 98% identity and, most preferably, 99% identity with any of the referred to sequences.

Calculation of percentage identities between different amino acid/polypeptide/nucleic acid sequences may be carried out as follows. A multiple alignment is first generated by the ClustalX program (pairwise parameters: gap opening 10.0, gap extension 0.1, protein matrix Gonnet 250, DNA matrix IUB; multiple parameters: gap opening 10.0, gap extension 0.2, delay divergent sequences 30%, DNA transition weight 0.5, negative matrix off, protein matrix gonnet series, DNA weight IUB; Protein gap parameters, residue-specific penalties on, hydrophilic penalties on, hydrophilic residues GPSNDQERK, gap separation distance 4, end gap separation off). The percentage identity is then calculated from the multiple alignment as (N/T)*100, where N is the number of positions at which the two sequences share an identical residue, and T is the total number of positions compared. Alternatively, percentage identity can be calculated as (N/S)*100 where S is the length of the shorter sequence being compared. The amino acid/polypeptide/nucleic acid sequences may be synthesised *de novo*, or may be native amino acid/polypeptide/nucleic acid sequence, or a derivative thereof.

Alternatively, a substantially similar nucleotide sequence will be encoded by a sequence which hybridizes to any of the nucleic acid sequences referred to herein or their complements under stringent conditions. By stringent conditions, we mean the nucleotide hybridises to filter-bound DNA or RNA in 6x sodium chloride/sodium citrate (SSC) at approximately 45°C followed by at least one wash in 0.2x SSC/0.1% SDS at approximately 5-65°C. Alternatively, a substantially similar polypeptide may differ by at least 1, but less than 5, 10, 20, 50 or 100 amino acids from the peptide sequences according to the present invention.

Due to the degeneracy of the genetic code, it is clear that any nucleic acid sequence could be varied or changed without substantially affecting the sequence of the protein encoded thereby, to provide a functional variant thereof. Suitable nucleotide variants are those having a sequence altered by the substitution of different codons that encode the same amino acid within the sequence, thus producing a silent change. Other suitable variants are those having homologous nucleotide sequences but comprising all, or portions of, sequence which are altered by the substitution of different codons that encode an amino acid with a side chain of similar biophysical properties to the amino acid it substitutes, to produce a conservative change. For

example small non-polar, hydrophobic amino acids include glycine, alanine, leucine, isoleucine, valine, proline, and methionine. Large non-polar, hydrophobic amino acids include phenylalanine, tryptophan and tyrosine. The polar neutral amino acids include serine, threonine, cysteine, asparagine and glutamine. The positively charged (basic) amino acids include lysine, arginine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

The accurate alignment of protein or DNA sequences is a complex process which has been investigated in detail by a number of researchers. Of particular importance is the trade-off between optimal matching of sequences and the introduction of gaps to obtain such a match. In the case of proteins, the means by which matches are scored is also of significance. The family of PAM matrices (e.g., Dayhoff, M. et al., 1978, Atlas of protein sequence and structure, Natl. Biomed. Res. Found.) and BLOSUM matrices quantitate the nature and likelihood of conservative substitutions and are used in multiple alignment algorithms, although other, equally applicable matrices will be known to those skilled in the art. The popular multiple alignment program ClustalW, and its windows version ClustalX (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882) are efficient ways to generate multiple alignments of proteins and DNA.

Frequently, automatically generated alignments require manual alignment, exploiting the trained user's knowledge of the protein family being studied, e.g., biological knowledge of key conserved sites. One such alignment editor programs is Align (http://www.gwdg.de/~dhepper/download/; Hepperle, D., 2001: Multicolor Sequence Alignment Editor. Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany), although others, such as JalView or Cinema are also suitable.

Calculation of percentage identities between proteins occurs during the generation of multiple alignments by Clustal. However, these values need to be recalculated if the alignment has been manually improved, or for the deliberate comparison of two sequences. Programs that calculate this value for pairs of protein sequences within an alignment include PROTDIST within the PHYLIP phylogeny

package (Felsenstein; http://evolution.gs.washington.edu/ phylip.html) using the "Similarity Table" option as the model for amino acid substitution (P). For DNA/RNA, an identical option exists within the DNADIST program of PHYLIP.

Other modifications in protein sequences are also envisaged and within the scope of the claimed invention, i.e. those which occur during or after translation, e.g. by acetylation, amidation, carboxylation, phosphorylation, proteolytic cleavage or linkage to a ligand.

It will be appreciated that polypeptides, derivatives and analogues according to the invention represent favourable agents to be administered by techniques involving cellular expression of nucleic acid sequences encoding such molecules. Such methods of cellular expression are particularly suitable for medical use in which the therapeutic effects of the polypeptides, derivatives and analogues are required over a prolonged period.

Thus according to a sixth aspect of the present invention there is provided a nucleic acid sequence according to the fifth aspect of the invention for use as a medicament.

According to a further aspect, there is provided use of the nucleic acid, for the preparation of medicament for treating a bacterial infection.

The nucleic acid may preferably be an isolated or purified nucleic acid sequence. The nucleic acid sequence may preferably be a DNA sequence.

The nucleic acid sequence may further comprise elements capable of controlling and/or enhancing its expression. The nucleic acid molecule may be contained within a suitable vector to form a recombinant vector. The vector may for example be a plasmid, cosmid or phage. Such recombinant vectors are highly useful as delivery systems for transforming cells with the nucleic acid molecule.

Recombinant vectors may also include other functional elements. For instance, recombinant vectors can be designed such that the vector will autonomously replicate in

the cell. In this case elements that induce nucleic acid replication may be required in the recombinant vector. Alternatively, the recombinant vector may be designed such that the vector and recombinant nucleic acid molecule integrates into the genome of a cell. In this case nucleic acid sequences, which favour targeted integration (e.g. by homologous recombination) are desirable. Recombinant vectors may also comprise DNA coding for genes that may be used as selectable markers in the cloning process.

The recombinant vector may also further comprise a promoter or regulator to control expression of the gene as required.

The nucleic acid molecule may (but not necessarily) be one, which becomes incorporated in the DNA of cells of the subject being treated. Undifferentiated cells may be stably transformed leading to the production of genetically modified daughter cells (in which case regulation of expression in the subject may be required e.g. with specific transcription factors or gene activators). Alternatively, the delivery system may be designed to favour unstable or transient transformation of differentiated cells in the subject being treated. When this is the case, regulation of expression may be less important because expression of the DNA molecule will stop when the transformed cells die or stop expressing the protein (ideally when the required therapeutic effect has been achieved).

The delivery system may provide the nucleic acid molecule to the subject without it being incorporated in a vector. For instance, the nucleic acid molecule may be incorporated within a liposome or virus particle. Alternatively a "naked" nucleic acid molecule may be inserted into a subject's cells by a suitable means, e.g. direct endocytotic uptake.

The nucleic acid molecule may be transferred to the cells of a subject to be treated by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the nucleic acid molecule, viral vectors (e.g. adenovirus) and means of providing direct nucleic acid uptake (e.g. endocytosis) by application of the nucleic acid molecule directly.

It will be appreciated that the polypeptides, agents, nucleic acids or derivatives according to the present invention may be used in a monotherapy (i.e. use of polypeptides, agents, nucleic acids or derivatives according to the invention alone to prevent and/or treat a bacterial infection). Alternatively, polypeptides, agents, nucleic acids or derivatives according to the invention may be used as an adjunct, or in combination with known therapies.

In accordance with a seventh aspect of the invention, there is provided a method of preventing and/or treating a bacterial infection, comprising administering to a subject in need of such treatment a therapeutically effective amount of a polypeptide, derivative, or analogue or nucleic acid according to the invention.

Polypeptides, agents, nucleic acids or derivatives according to the invention may be combined in compositions or compounds having a number of different forms depending, in particular, on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, ointment, cream, gel, hydrogel, aerosol, spray, micelle, transdermal patch, liposome or any other suitable form that may be administered to a person or animal. It will be appreciated that the vehicle of the composition of the invention should be one which is well tolerated by the subject to whom it is given, and preferably enables delivery of the polypeptides, agents, nucleic acids or derivatives to the brain. It is preferred that the polypeptides, agents, nucleic acids or derivatives according to the invention be formulated in a manner that permits their passage across the blood brain barrier.

Compositions comprising polypeptides, agents, nucleic acids or derivatives according to the invention may be used in a number of ways. For instance, systemic administration may be required in which case the compound may be contained within a composition that may, for example, be ingested orally in the form of a tablet, capsule or liquid. Alternatively, the composition may be administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion). The compounds may be administered by inhalation (e.g. intranasally).

Polypeptides, agents, nucleic acids or derivatives may also be incorporated within a slow or delayed release device. Such devices may, for example, be inserted on or under the skin, and the compound may be released over weeks or even months. Such devices may be particularly advantageous when long term treatment with a polypeptide, agent, nucleic acid or derivative according to the invention is required and which would normally require frequent administration (e.g. at least daily injection).

It will be appreciated that the amount of a polypeptide, agent, nucleic acid or derivative that is required is determined by its biological activity and bioavailability which in turn depends on the mode of administration, the physicochemical properties of the polypeptide, agent, nucleic acid or derivative employed and whether the polypeptide, agent, nucleic acid or derivative is being used as a monotherapy or in a combined therapy. The frequency of administration will also be influenced by the above-mentioned factors and particularly the half-life of the polypeptide, agent, nucleic acid or derivative within the subject being treated.

Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular polypeptide, agent, nucleic acid or derivative in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials, etc.), may be used to establish specific formulations of polypeptides, agents, nucleic acids or derivatives according to the invention and precise therapeutic regimes (such as daily doses of the polypeptides, agents, nucleic acids or derivatives and the frequency of administration).

Generally, a daily dose of between 0.01 μg/kg of body weight and 0.5 g/kg of body weight of polypeptides, agents, nucleic acids or derivatives according to the invention may be used for the prevention and/or treatment of a viral infection,

depending upon which specific polypeptide, agent, nucleic acid or derivative is used. More preferably, the daily dose is between 0.01 mg/kg of body weight and 200 mg/kg of body weight, and most preferably, between approximately 1mg/kg and 100 mg/kg.

Daily doses may be given as a single administration (e.g. a single daily injection). Alternatively, the polypeptide, agent, nucleic acid or derivative used may require administration twice or more times during a day. As an example, polypeptides, agents, nucleic acids or derivatives according to the invention may be administered as two (or more depending upon the severity of the condition) daily doses of between 25 mg and 7000 mg (i.e. assuming a body weight of 70kg). A patient receiving treatment may take a first dose upon waking and then a second dose in the evening (if on a two dose regime) or at 3 or 4 hourly intervals thereafter. Alternatively, a slow release device may be used to provide optimal doses to a patient without the need to administer repeated doses.

This invention provides a pharmaceutical composition comprising a therapeutically effective amount of a polypeptide, agent, nucleic acid or derivative according to the invention and optionally a pharmaceutically acceptable vehicle. In one embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.01 mg to about 800 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.01 mg to about 500 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.01 mg to about 250 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.1 mg to about 60 mg. In another embodiment, the amount of the polypeptide, agent, nucleic acid or derivative is an amount from about 0.1 mg to about 20 mg.

This invention provides a process for making a pharmaceutical composition comprising combining a therapeutically effective amount of a polypeptide, agent, nucleic acid or derivative according to the invention and a pharmaceutically acceptable vehicle. A "therapeutically effective amount" is any amount of a polypeptide, agent, nucleic acid or derivative according to the invention which, when

administered to a subject provides prevention and/or treatment of a viral infection. A "subject" is a vertebrate, mammal, domestic animal or human being.

A "pharmaceutically acceptable vehicle" as referred to herein is any physiological vehicle known to those of ordinary skill in the art useful in formulating pharmaceutical compositions.

In a preferred embodiment, the pharmaceutical vehicle is a liquid and the pharmaceutical composition is in the form of a solution. In another embodiment, the pharmaceutically acceptable vehicle is a solid and the composition is in the form of a powder or tablet. In a further embodiment, the pharmaceutical vehicle is a gel and the composition is in the form of a cream or the like.

A solid vehicle can include one or more substances which may also act as flavouring agents, lubricants, solubilisers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the vehicle is a finely divided solid that is in admixture with the finely divided active polypeptide, agent, nucleic acid or derivative. In tablets, the active polypeptide, agent, nucleic acid or derivative is mixed with a vehicle having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active polypeptide, agent, nucleic acid or derivative. Suitable solid vehicles include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid vehicles are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active polypeptide, agent, nucleic acid or derivative can be dissolved or suspended in a pharmaceutically acceptable liquid vehicle such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid vehicle can contain other suitable pharmaceutical additives such as solubilisers, emulsifiers, buffers, preservatives, sweeteners, flavouring agents, suspending agents, thickening agents, colours, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid vehicles for oral and

parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the vehicle can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid vehicles are useful in sterile liquid form compositions for parenteral administration. The liquid vehicle for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by for example, intramuscular, intrathecal, epidural, intraperitoneal, intravenous and particularly subcutaneous, intracerebral or intracerebroventricular injection. The polypeptide, agent, nucleic acid or derivative may be prepared as a sterile solid composition that may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium. Vehicles are intended to include necessary and inert binders, suspending agents, lubricants, flavourants, sweeteners, preservatives, dyes, and coatings.

Polypeptides, agents, nucleic acids or derivatives according to the invention can be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like.

Polypeptides, agents, nucleic acids or derivatives according to the invention can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

In addition to administering the said polypeptides, agents, nucleic acids or derivatives according to the invention to a patient, they may be used to coat at least part of a biomaterial, for example, a medical device.

Therefore, in a further aspect there is provided a method of preventing and/or treating a bacterial infection, comprising coating at least part of a biomaterial with a therapeutically effective amount of a polypeptide, derivative, or analogue or nucleic acid according to any preceding claim.

Examples of medical devices include lenses, catheters, stents, wound healing dressings, contraceptives or surfaces for use in medical environments, including surfaces of equipment for use in operating theatres or as a routine constituent of physiological solutions (for example as a constituent of physiological saline). However, it will be appreciated that the peptides may be administered to any surface, which is prone to a bacterial infection. In preferred embodiment, the peptides may be included in saline solution used to store contact lenses.

The highly positively charged nature of these peptides make a most preferred usage thereof to coating medical devices and other biomaterials to prevent growth of broad categories of bacteria.

Preferably, coating of the biomaterial may be carried out by preparing an aqueous solution at an appropriate pH and temperature of the said polypeptides, agents, nucleic acids or derivatives according to the invention, and exposing the biomaterial to the said solution for sufficient time to allow immobilisation or absorption of a suitable quantity of the peptides to the surface thereof.

In addition, the inventors have found that contact lenses, when pre-incubated with the polypeptides according to the invention, become resistant to bacterial infection as is illustrated in Figures 2, 3 and 4.

Furthermore, a combination of several polypeptides, agents, nucleic acids or derivatives according to the invention would be another preferred method of preventing or treating a broad range of bacterial infections. For example, it may be

preferred to treat a bacterial infection with a combination of peptides independently selected from a group consisting of GIN 1p, GIN 7, GIN 32, GIN 33 and GIN 34. However, it will be appreciated that different combinations of peptides can be used to treat different bacterial infections.

Furthermore, the polypeptides, agents, nucleic acids or derivatives according to the invention may be used to minimise, prevent or treat bacterial infections or bacterial growth, by use as, or in conjunction with, a preservative. Hence, the polypeptides, agents, nucleic acids or derivatives may be used as a preservative in foodstuffs. In addition, the polypeptides, agents, nucleic acids or derivatives may be used to minimise or prevent bacterial growth in cultures, for example, in tissue culture work, either to supplement, or to replace antibiotics. Furthermore, the polypeptides, agents, nucleic acids or derivatives may be used to treat any mammal, for example, human, livestock, pets, and may be used in other veterinary applications.

The inventors believe that the peptides in accordance with the invention are novel.

According to an eighth aspect, there is provided a polypeptide, derivative or analogue thereof comprising a tandem repeat of $apoE_{141-149}$ of SEQ ID No.6 or a truncation thereof, characterised in that at least one Leucine (L) residue of SEQ ID No. 6 is replaced by a Tryptophan (W) or Tyrosine (Y) residue or derivatives thereof.

According to a ninth aspect, there is provided a polypeptide, derivative or analogue thereof comprising a tandem repeat of a peptide derived from a Heparan Sulphate Proteoglycan (HSPG) receptor binding region of apolipoprotein B or apolipoprotein E, or a truncation thereof.

It will be appreciated that the peptides according to the above aspects can be combined with the peptides according to the eighth and ninth aspects described herein.

All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed,

may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

Embodiments of the invention will now be further described, by way of example only, with reference to the following Example and figures in which:-

Figure 1 illustrates the level of growth of *Pseudomonas aeruginosa* after exposure of a bacterial inoculum to various concentrations of peptide GIN 1p in accordance with the present invention, or of a dummy dilution of PBSA.

Figure 2 is a bar graph illustrating growth of *Pseudomonas aeruginosa* overnight on Johnson and Johnson Acuvue contact lenses following pre-treatment with peptide GIN 1p, or a control treatment;

Figure 3 is a photograph showing the appearance of two GIN1p-treated and two control-treated contact lenses positioned in four wells of a 24-well plate after overnight incubation;

Figure 4 is a light micrograph showing the appearance of the surface of a (i) control-treated or a (ii) GIN1p-treated contact lens after overnight incubation;

Figure 5 illustrates MTT reduction in cells treated with various levels of GIN peptides for 48hours; and

Figures 6A, 6B & 6C illustrate the lack of genotoxic effects of peptides GIN 1p, GIN 33 and GIN 34, respectively, as indicated by a yeast genotoxicity assay.

Example 1

Antibacterial Efficacy Assessment

Bacterial Stocks - ATCC derived stock bacteria were obtained from Oxoid Limited. *Pseudomonas aeruginosa* (ATCC strain 9027) or *Staphylococcus aureus* (ATCC strain 6538P) were obtained in 'Cultiloop' format. Stocks were prepared by inoculating 20 ml of LB broth with a single Cultiloop, and incubating overnight at 37^0C. Cells present after overnight incubation were harvested by removing larger aggregates by centrifugation (3000g, 10 minutes), and withdrawing 16 ml of the supernatant, which was likely to contain mainly planktonic bacteria. To this, 4 ml of sterile glycerol was added (the latter having been sterilised by autoclaving). The resultant suspension was divided between 15x 1 ml cryovials before freezing at – 85^0C.

Antibacterial Assay

Preliminary experiments were carried out to estimate the amount to dilute bacterial stocks which when on addition of 25µl of diluted stock to 100 µl of fresh LB broth would result in bacteria in log phase growth after overnight incubation at 37^0C. This was found to correspond to around 40,000 cfu per well at inoculation.

Peptides

Peptides were obtained in lyophilised form from a commercial supplier (AltaBioscience, University of Birmingham), and were produced at 5 micromole scale. N-terminals were protected by addition of an acetyl group, and the C-terminals were protected by addition of an amide group. Small quantities of peptide were weighed in sterile Eppendorf tubes, before addition of sufficient PBSA to produce a 0.4 mM stock solution, which was frozen at -85°C in aliquots.

Antibacterial Efficacy against *Staphylococcus aureus*

The peptides in accordance with the invention were examined for antibacterial efficacy against *Staphylococcus aureus*. The results are shown in Table 1. To test the efficacy of peptides, dilutions of test peptides or PBS (for use as a control) were prepared in LB broth, and 100µl aliquots of these placed in 96-well plates. A vial of planktonic bacterial stock was thawed in the 37^0C incubator and diluted to the level

previously determined which would produce log phase growth after overnight incubation (see above). 25 µl of this bacterial dilution was then added to the test wells (some wells containing LB broth without peptide were left without any bacterial inoculation, these being used as a blank). After overnight growth, the concentration of bacteria in individual wells was assessed by measuring absorbance at 620nm, in a microplate spectrophotometer. The average absorbance of wells for specific concentrations of peptides was calculated, and from this the percentage inhibition (relative to untreated wells) was also calculated and plotted against concentration (along with an estimate of error on that % inhibition). Finally, the approximate concentration of peptide inhibiting bacterial growth by 50% was estimated from this figure to calculate the IC50 value.

The peptides according to the invention, which were tested are shown in Table 1.

Table 1. Activity of apoE-derived peptides against *Staphylococcus aureus*

Peptide Reference	IC50 (µM)	Sequence
GIN 34	7	WRKWRKRWWLRKLRKRLL
GIN 33	9.5	WRWRKRWRKWRWRKRWRK
GIN 32	10	WRKWRKRWRKWRKR
GIN 1p	13	LRKLRKRLLLRKLRKRLL
GIN 7	19	WRKWRKRWWWRKWRKRWW
GIN 41	33	YRKYRKRYYYRKYRKRYY

Sequences where no activity could be detected:-

Peptide Reference	Sequence
GIN 6	ERKERKREEERKERKREE
GIN 22	DWLKAFYDKVAEKLKEAF
GIN 39	ARKARKRAAARKARKRAA
GIN 40	MRKMRKRMMMRKMRKRMM

Antibacterial Efficacy against *Pseudomonas aeruginosa*

The peptides in accordance with the invention were also examined for antibacterial efficacy against *Pseudomonas aeruginosa* using the same technique as described

above to calculate the IC50 values. The peptides according to the invention, which were tested are shown in Table 2.

Table 2. Activity of apoE-derived peptides against *Pseudomonas aeruginosa*

Peptide Reference	IC50 (uM)	Sequence
GIN 1p	3	LRKLRKRLLLRKLRKRLL
GIN 34	3	WRKWRKRWWLRKLRKRLL
GIN 7	5	WRKWRKRWWWRKWRKRWW
GIN 32	5	WRKWRKRWRKWRKR
GIN 2	8.5	LRKRLLLRKLRKRLL
GIN 8	9.2	LRKLRKRLRKLRKR
GIN 33	12	WRWRKRWRKWRWRKRWRK
GIN 11	15	QSTEELRVRLASHLRKLRKRLL

Sequences where no activity could be detected:

Peptide Reference	Sequence
GIN 6	ERKERKREEERKERKREE
GIN 10	RLLRLLRLLRLLRLLRLL
GIN 12	LRKLRKRLLRDADDLQKRLA
GIN 22	DWLKAFYDKVAEKLKEAF
GIN 28	LRKEKKRLLLRKEKKRLL
GIN 39	ARKARKRAAARKARKRAA
GIN 40	MRKMRKRMMMRKMRKRMM
GIN 43	LRYLRYRLLLRYLRYRLL

Referring to Figure 1, there is shown the level of growth of *Pseudomonas aeruginosa* after exposure of a bacterial inoculum to various concentrations of peptide GIN 1p in accordance with the present invention, or of a dummy dilution of PBSA. Points were obtained by dividing the average for six treated wells by the average for six untreated wells, and bars show an estimate of the error for these calculated values. Figure 1 clearly shows the antibacterial efficacy of GIN 1p (SEQ ID No.6) at a concentration of approx. 4μM, and over, and indicates that GIN 1p has an IC50 concentration of approximately 3μM.

In summary, the peptides according to the invention exhibit antibacterial activity against both *S. aureus*, and *P. aeruginosa*.

Hence, the peptides according to the invention may themselves be useful for the prevention and treatment of bacterial infection, as may peptoids derivatives of these compounds. One method of administration of the agents is by coating medical devices prone to developing such infections. Examples of medical devices include lenses, catheters, stents, wound healing dressings, contraceptives or surfaces for use in medical environments, including surfaces of equipment for use in operating theatres or as a routine constituent of physiological solutions (for example as a constituent of physiological saline). However, it will be appreciated that the peptides may be administered to any surface which is prone to a bacterial infection.

Coating a surface can be carried out by preparing a concentrated aqueous solution (for example 200μM) at an appropriate pH (for example pH7.4) of the polypeptides according to the invention, and then exposing the surface to the aqueous solution at a suitable temperature (for example 37^0C) for sufficient time (for example 2 hours) to allow immobilisation or absorption of a suitable quantity of the peptides to the surface thereof.

In addition, the compounds may also be useful when administered as systemic drugs to treat bacterial infection, either when applied as a topical formulation, applied by inhalation, administered by oral ingestion, or introduced by intravenous or other methods of injection.

Experiment to test Antibacterial Efficacy on contact lenses

The inventors have found that contact lenses, when pre-incubated with the peptides in accordance with the invention, become resistant to bacterial infection. Referring to Figure 2, there is shown a bar graph illustrating growth of *Pseudomonas aeruginosa* overnight on Johnson and Johnson Acuvue contact lenses following pre-treatment with peptide GIN 1p, or a control treatment. Growth was measured by examination by light microscopy, and by assessing metabolic activity of any bacteria present using MTT reduction. The data illustrates the efficacy of the antibacterial nature of the

peptides according to the invention when applied to contact lenses. Antibacterial activity was assessed by measuring MTT reduction.

Lenses were placed in 24-well plates before treatment with 75µl of 250µM GIN-1p in PBS (pH7.4) or with 75µl of PBS and incubated for 2 hours at 37^0C, before removal of these solutions from the lens by aspiration. A further 1 ml of PBS was then added to the wells to wash away any loosely attached peptide, before aspiration of this wash solution. Finally, 1ml of 20% (v/v) LB broth in PBS was added to each well, the latter containing 10^4 challenge organisms (an amount which approximates to the likely level of challenge which would be appropriate for the eye). Calculations suggest that even if aspiration of the initial peptide solution or wash solution was not complete, the concentration of peptide carried over and still present in the system in solution (i.e. merely as a dilution of the original peptide stock) would be less than 200 nM, which would be too low on its own to inhibit bacterial infection.

The plates were returned to the incubator at 37^0C overnight. After approximately 15 hr incubation, some of the lenses were exposed to MTT solution at 2mg/ml, and incubated for a further one hour at 37^0C. The remaining lenses were examined both using a light microscope (Olympus IX70) or examined without magnification. The MTT treated lenses were removed from the MTT solution after the further incubation, and placed in 1ml DMSO, to solubilise any blue formazan crystals produced by due to the metabolic activity of any bacteria present. The degree of formazan production was assessed by measuring absorbance at 540nm for aliquots of these solutions; these values are shown in Figure 2, and represent the average for four lenses, with the bars showing standard deviation.

Referring to Figure 3, there is shown a photograph showing the appearance of two GIN1p-treated contact lenses (right hand side of Figure 3) and two control-treated contact lenses (left hand side of Figure 3) positioned in four wells of a 24-well plate after overnight incubation. The Figure clearly shows that the two GIN1p-treated contact lenses are less prone to developing a bacterial infection. The reaction solution containing the two control lenses, which were not pre-treated with a peptide according to the invention, are cloudy, indicating that bacteria are growing therein. However, the

solutions containing the two GIN1p pre-treated contact lenses are colourless, indicating that the majority of any bacterial growth has been minimised.

Referring to Figure 4, there is shown a light micrograph showing the appearance of the surface of the (i) control-treated contact lens, and the (ii) GIN1p-treated contact lens after overnight incubation. Figure 4 clearly shows that the control-treated lens is covered with bacterial growth, whereas the GIN1p-treated lens show minimal bacterial growth. Hence, the peptide GIN1p, in accordance with the invention, clearly exhibits antibacterial activity.

Hence, the inventors suggest that any method of immobilisation of these peptides on lenses in an active anti-microbial form will reduce the risk of microbe related adverse reactions in contact lens wearers, such as microbial keratitis.

Toxicity assays

Referring to Figure 5, there is shown MTT reduction in cells treated with various levels of GIN1p and GIN 16 for 48hours.

African Green Monkey Kidney (Vero) cells were maintained in Eagle's minimum essential medium with Earle's salt (EMEM) and supplemented with 10% fetal calf serum (heat-inactivated), 4 mM L-glutamine, and 1% (v/v) non-essential amino acids, plus penicillin and streptomycin (100 IU/mg and 100 mg/ml, respectively) (maintenance medium referred to as 10% EMEM). The cells were incubated at 37°C in a humidified atmosphere of air with 5% CO_2. On harvesting, monolayers were washed in phosphate-buffered saline (PBS), and dislodged by incubating with trypsin in PBS for 30min, before inactivating trypsin by addition of an equal volume of 10% EMEM and centrifuging at 500g (5 min, 4°C).

Vero cells were seeded in 96-well plates at 30,000 cells per well in 100µl of 10% EMEM. After overnight growth, medium was gently aspirated and replaced with 10% EMEM containing various concentrations of peptides or in some cases just 10% EMEM alone. Cells were returned to the incubator for 48hr, before addition of 25µl of 1.5mg/ml MTT solution (prepared in 0.5% EMEM, then filtered though a sterile 0.22µm filter). The plates were then returned to the incubator for one hour. Finally,

medium was removed from wells, and any blue formazan crystals solubilised by addition of 100 μl of dimethylsulphoxide (DMSO). Absorbance of resulting solutions was then measured at 570nm, and toxic effect expressed as a percentage of the control value for each peptide concentration. Where possible, the EC50 was calculated from plots of toxic effect against peptide concentration. Fortunately, no evidence of toxicity was found for the cell line tested, using peptide at 40μM exposed to cells for 2 days.

Genotoxicity

Additionally, the inventors have found that despite the high numbers of positive charges on these peptides, they have little or no genotoxic activity, as revealed by the Gentronix GreenScreen GC genotoxicity assay (Gentronix Ltd., 72 Sackville St, Manchester, M60 1QD, UK), which was used to test the peptides GIN1p, GIN 33 and GIN 34, and as summarised in Figures 6A-6C.

A liquid handling robot is used to produce a serial dilution series of each test compound in a 96-well, black microplate with an optically clear base. Each dilution of the test compound is combined with an equal volume of a specialised growth medium containing *Saccharomyces cerevisiae* yeast cells (strain GenT01: see Mutation Research, 2000, 464 297-308 for details of the properties of these modified strains) to give a fixed final volume and concentration of cells. In addition, a series of genotoxic and cytotoxic standards and non-toxic controls are run to provide internal quality control. The plates are covered with a breathable membrane and incubated overnight, without shaking, at 25°C.

After incubation, the microplates are uncovered and data collected using a Tecan Ultra 384 microplate reader. This instrument provides measurements of light absorbance and fluorescence in microplates; a standard fluorescein filter set (excitation 485/25 and emission 535/25) is used. Absorbance is read at 620nm and is proportional to cell proliferation (which is lowered by toxic analytes). Fluorescence is proportional to the activity of the DNA repair system (increased by genotoxic analytes). The yeast cultures used are a genetically modified strain that express a green fluorescent protein whenever the cells carry out DNA damage repair. Fluorescence is normalised to the absorbance signal to correct for variation in cell

yield caused by any cytolytic activity of the test compounds against the yeast cells. This produces a "fluorescence per cell" measurement termed "brightness". In addition to the Gentronix Assay yeast strain (GenT01), a control *Saccharomyces cerevisiae* strain (GenC01) is also used, which is identical except for its ability to produce green fluorescent protein is disabled. This strain is used to provide a control to allow correction for substances which may be either autofluorescent themselves or which induce an autofluorescence in the yeast cells. The data collected is simply transferred into a Excel template where result evaluation sheets are automatically produced. The sheets contain the processed results in both a graphical and tabulated format, with automatic assessment of the result, i.e. positive or negative, and calculation of the lowest effective concentration (LEC), for both genotoxicity and cytotoxicity.

Referring to Figures 6A-6C, there are shown yeast genotoxicity assay obtained for peptides GIN 1p, GIN 33 and GIN34, respectively. The control strain (GenC01) behaved as expected with little autofluorescence being apparent in this strain in the presence of the peptides, though nonetheless the data obtained using the test strain (GenT01) were corrected to take account of this minimal background. Figures 6A-6C show that all three peptides failed to produce any genotoxic response in the test yeast strain (GenT01), with values for peptide concentrations up to and including 100μM clearly failing to reach the threshold required for genotoxicity to be indicated.

In conclusion, the results indicate the antibacterial efficacy of the peptides in accordance with the present invention. In particular, the peptides GIN 34 (SEQ ID No. 12), GIN 33 (SEQ ID No. 16), GIN 32 (SEQ ID No. 8), GIN 1p (SEQ ID No. 6), GIN 7 (SEQ ID No. 7), GIN 41 (SEQ ID No. 10), GIN 2 (SEQ ID No. 3), GIN 8 (SEQ ID No. 11), and GIN 11 (SEQ ID No. 4) exhibited antibacterial activity.

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Leu Leu

CLAIMS

1. A peptide, derivative or analogue thereof comprising an amino acid sequence of SEQ ID No.3 or SEQ ID No.4, for use as a medicament.

2. Use of a tandem repeat of a peptide, derivative or analogue thereof, which peptide is derived from a Heparan Sulphate Proteoglycan (HSPG) receptor binding region of apolipoprotein B or apolipoprotein E, or a truncation thereof, for the manufacture of a medicament for treating a bacterial infection.

3. Use according to claim 2, wherein the peptide, derivative or analogue thereof is derived from an apolipoprotein B LDL receptor binding domain cluster B, or from an apolipoprotein E LDL receptor binding domain.

4. Use according to either claim 2 or 3, wherein the peptide, derivative or analogue thereof comprises a tandem repeat of $apoE_{133-162}$ of SEQ ID NO. 5, or a truncation thereof.

5. Use according to either claim 2 or 3, wherein the peptide, derivative or analogue thereof comprises a tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6, or a truncation thereof.

6. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises a tandem repeat of $apoE_{141-149}$ of SEQ ID NO. 6, characterised in that at least one Leucine (L) residue of SEQ ID No. 6 is replaced by a Tryptophan (W) or Tyrosine (Y) residue or derivatives thereof.

7. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: WRKWRKRWWWRKWRKRWW (SEQ ID No. 7).

8. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: WRKWRKRWRKWRKR (SEQ ID No. 8).

9. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: WRKWRKRWWLRKLRKRLL (SEQ ID No. 9).

10. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: YRKYRKRYYYRKYRKRYY (SEQ ID No. 10).

11. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: LRKLRKRLRKLRKR (SEQ ID No. 11).

12. Use according to claim 5, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: LRKRLLLRKLRKRLL (SEQ ID No.4).

13. Use according to either claim 2 or claim 3, wherein the peptide, derivative or analogue thereof comprises a tandem repeat of $apoB_{3359-3367}$ of SEQ ID No. 12, or a truncation thereof.

14. Use according to claim 13, wherein the peptide, derivative or analogue comprises a tandem repeat of $apoB_{3359-3367}$ of SEQ ID No.12 or a truncation thereof, characterised in that at least one amino acid residue has been replaced by a Tryptophan (W), Arginine (R) or Leucine (L) residue or derivative thereof.

15. Use according to claim 14, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: RTRKRGRRTRKRGR (SEQ ID No.13).

16. Use according to claim 14, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: LRKRKRLLRKRKRL (SEQ ID No.14).

17. Use according to claim 14, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: LRKRKRLRKLRKRKRLRK (SEQ ID No.15).

18. Use according to claim 14, wherein the peptide, derivative or analogue thereof comprises an amino acid sequence: WRWRKRWRKWRWRKRWRK (SEQ ID No.16).

19. Use of a peptide comprising $apoE_{128-149}$ of SEQ ID NO.2, or a truncation thereof, for the manufacture of medicament for the treatment of a bacterial infection.

20. Use according to claim 19, wherein the peptide, derivative or analogue comprises the amino acid sequence: QSTEELRVRLASHLRKLRKRLL.

21. Use according to any of claims 2-20, wherein the peptide, derivative or analogue thereof is effective against *Staphylococcus* or *Pseudomonadales*.

22. An agent capable of increasing the biological activity of a polypeptide, derivative or analogue according to any preceding claim, for use as a medicament.

23. Use of an agent capable of increasing the biological activity of a polypeptide, derivative or analogue according to any preceding claim, for the manufacture of a medicament for treating a bacterial infection.

24. A nucleic acid sequence encoding a polypeptide, derivative or analogue according to any preceding claim.

25. A nucleic acid according to claim 14, wherein the nucleic acid comprises a nucleotide sequence as set out as SEQ ID No.25, SEQ ID No.26, SEQ ID No.27, SEQ ID No.28, SEQ ID No.29, SEQ ID No.30, SEQ ID No.31, SEQ ID No.32, SEQ ID No.33, SEQ ID No.34, SEQ ID No.35, SEQ ID No.36, SEQ ID No.37, and SEQ ID No.38.

26. A nucleic acid sequence according to either claim 24 or claim 25, for use as a medicament.

27. Use of a nucleic acid according to either claim 24 or claim 25, for the preparation of medicament for treating a bacterial infection.

28. A method of preventing and/or treating a bacterial infection, comprising administering to a subject in need of such treatment a therapeutically effective amount of a polypeptide, derivative, or analogue or nucleic acid according to any preceding claim.

29. A method of preventing and/or treating a bacterial infection, comprising coating at least part of a biomaterial with a therapeutically effective amount of a polypeptide, derivative, or analogue or nucleic acid according to any preceding claim.

30. A method according to claim 29, wherein the biomaterial comprises a medical device.

Figure 1

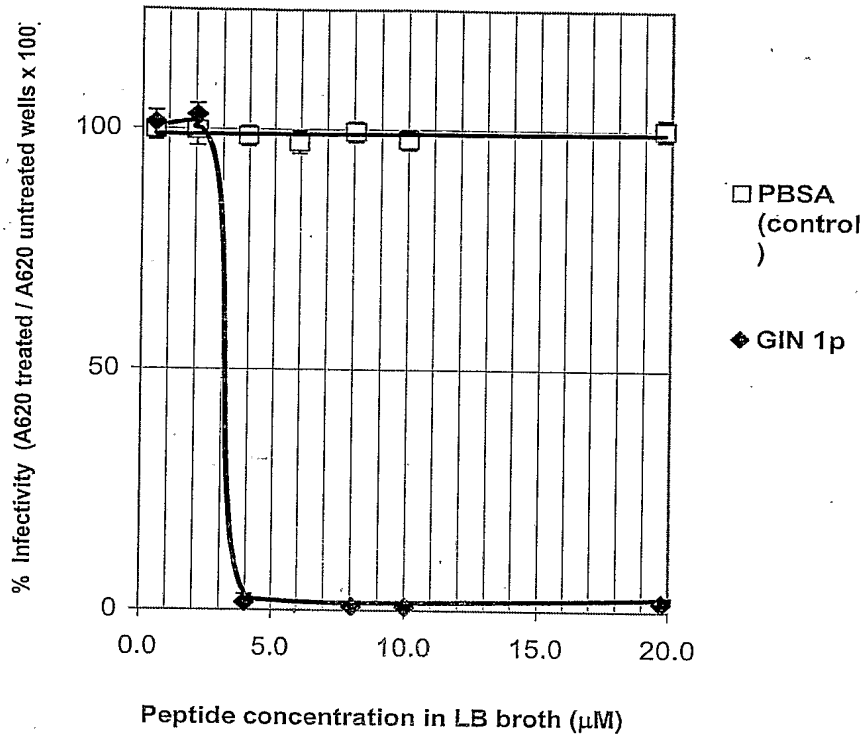

Figure 2

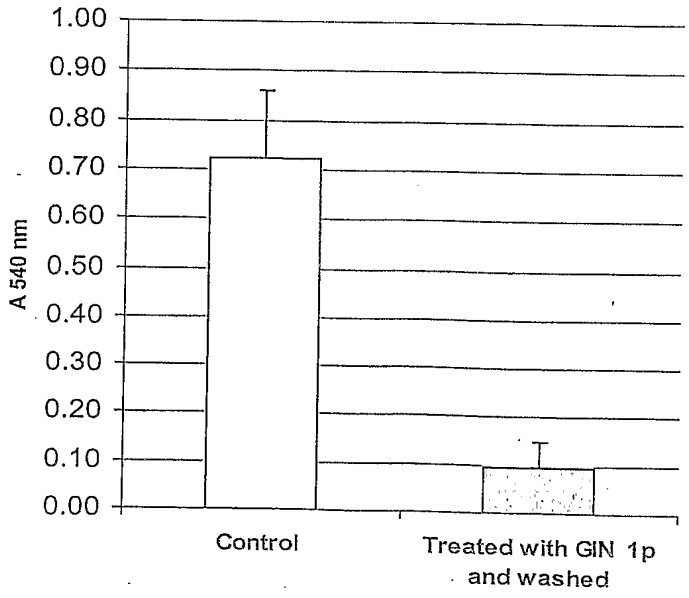

Figure 3

Control **GIN 1p treated**

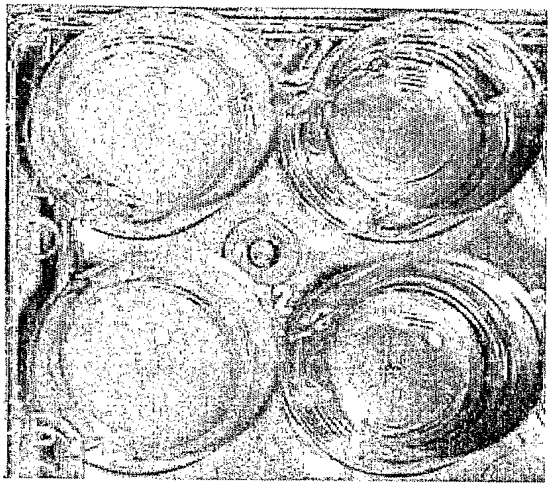

Figure 4

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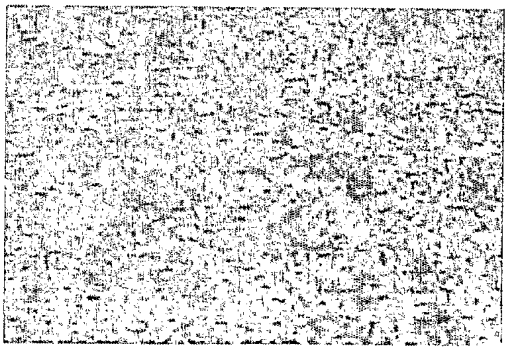

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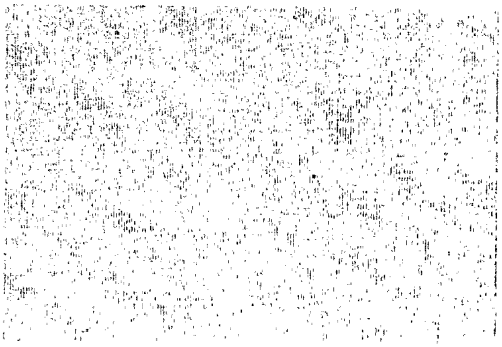

Figure 5

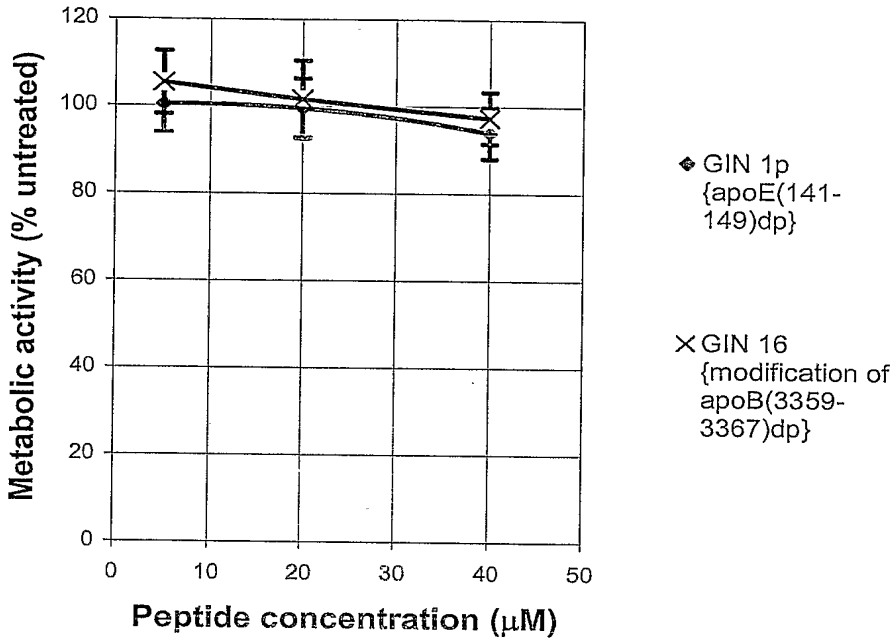

Figure 6A - GIN 1p

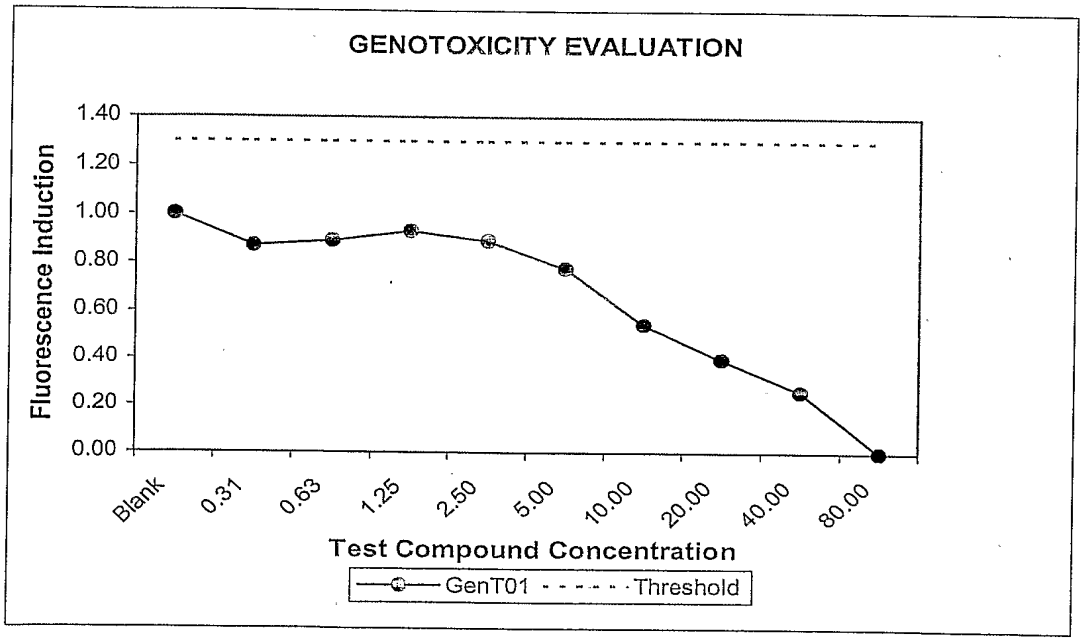

Figure 6B - GIN 33

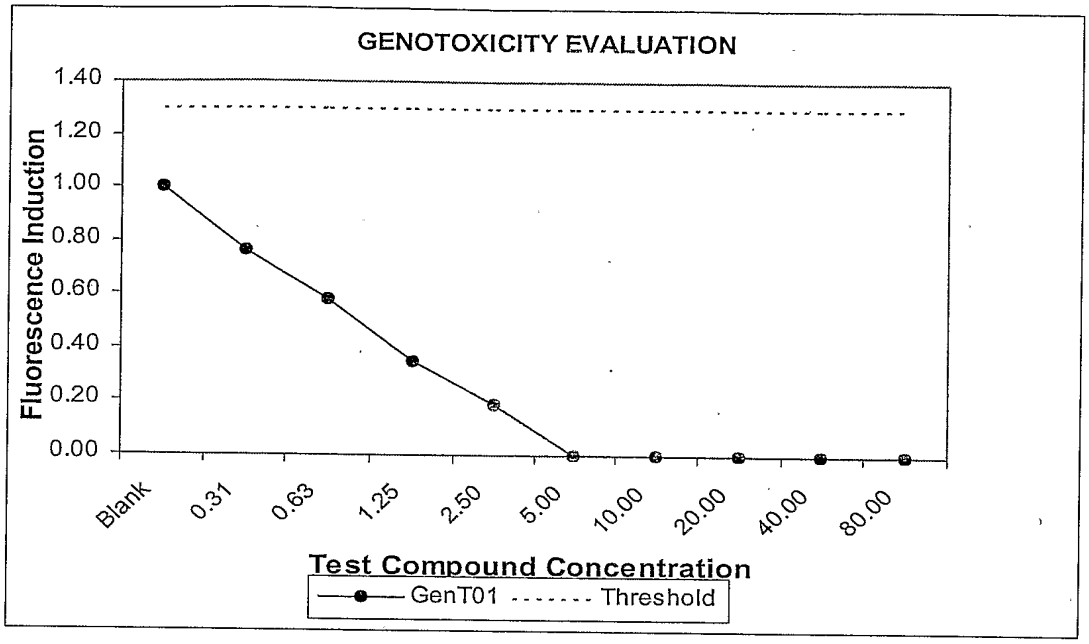

Figure 6C - GIN 34